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14. ABSTRACT
A main goal of this project is to determine a means of measuring receptive language in a more sensitive and accurate way than can currently be achieved in children with autism spectrum disorders (ASD) who are nonverbal, and neural markers have the potential to be more effective than behavioral measures in this population. This project will significantly contribute to the knowledge of underlying neurophysiology in language processing in ASD. We have successfully developed a technique to correct MEG data for subject movement during recording. This correction reduces signal loss due to movement, resulting in higher quality data. Additionally, we have created a video to send to parents of potential study participants, which describes the study procedures. This video was designed to increase subject comfort; based on reports from subjects, this approach appears to be successful in reducing anxiety surrounding the study visits. We have completed data collection with 5 subjects in the nonverbal ASD group, 2 subjects in the verbal ASD group, and 1 subject in the control group. The study is fully up and running, so we anticipate being able to complete study procedures with the originally planned 15 subjects per group by the end of Year 2.

15. SUBJECT TERMS
Autism spectrum disorders, magnetoencephalography (MEG), neural biomarkers, nonverbal

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Table of Contents

	Page
Introduction……..	**4**
Body……..	**5**
Key Research Accomplishments……..	**9**
Reportable Outcomes……	**10**
Conclusion……	**11**
References……	**12**
Appendix……	**13**

Introduction

Nonverbal individuals with autism spectrum disorders (ASD) are currently underrepresented in neuroimaging studies of ASD, leaving a critical gap in the understanding of language processing in ASD. The proposed project aims to address this gap by investigating auditory and language-specific processing in nonverbal children with ASD, in comparison to verbal children with ASD and typically developing healthy comparison children. Language and communication deficits are core symptoms of autism spectrum disorders and as many as a quarter of individuals with ASD are estimated to lack functional verbal skills[1]. The neural mechanisms involved in ASD are still largely unknown and even less is known for those who are minimally verbal. Determining points of processing at which deficits may lead to a lack of speech is critical for understanding language impairment in ASD. Assessing language comprehension in nonverbal children is challenging due to expressive language impairments; that is, given that this population has very low output of language, it is difficult to determine their level of verbal understanding. As such, the identification of biomarkers of language function that can be measured without a verbal response will be an important asset for evaluation of deficits and treatment outcome in this population. In this project, magnetoencephalography (MEG) is being utilized to measure brain activity during auditory and language processing in 3 groups: 1) nonverbal children with ASD, 2) verbal children with ASD, and 3) healthy comparison children. During MEG recording, participants complete two passive tasks not requiring a response. The first is a simple 5-minute auditory task during which participants hear a series of white noise stimuli[2, 3]. The second is a 15-minute language task (with brief breaks throughout), in which participants hear spoken words followed by pictures that either match (e.g., spoken word “lion”, picture of a lion) or do not match (e.g., spoken word “lion”, picture of a boat). Brain activity recorded during these tasks will provide three measures that have been implicated as biomarkers in ASD[2-4]: synchronized brain activity in the gamma frequency range (30-80 Hz), the M100 evoked field, and the M400 evoked field. The working hypothesis is that nonverbal children with ASD will show exaggerated deficits compared to those previously found in verbal children with ASD. This project has the potential to contribute to the knowledge of underlying neurophysiology in language processing in ASD, as well as identifying a more effective measure of language comprehension in nonverbal children with ASD.

Body

The following describes current progress on the approved elements of the Statement of Work (SOW) that fall within the scope of Year 1 (9/01/13-8/31/14) of the award:

STATEMENT OF WORK (SOW)

All work is performed at the University of Colorado Anschutz Medical Campus.

Task 1. **IRB Approval (prior to award commencement)**

a. Approval of human subjects' protocol with Colorado Multiple Institution Review Board. Application will be submitted 4 months prior to the award start date.
b. Approval of human subjects' protocol with Department of Defense (independent review from local IRB).

Progress on Task 1:
a. IRB approval was obtained prior to award commencement, in March 2013, through the Colorado Multiple Institutional Review Board (COMIRB) at the University of Colorado Anschutz Medical Campus (protocol #13-0204). A continuing review for this protocol was submitted to COMIRB and approved in March 2014, currently valid until March 2015.

b. Following COMIRB approval, the protocol was submitted to and approved by the U.S. Army Medical Research and Materiel Command (USAMRMC) Human Research Protections Office (HRPO), HRPO Log Number A-17777. The continuing review was also submitted to and approved by HRPO following COMIRB approval.

Task 2. **Assessment Preparation, Month 1**

a. Purchase behavioral assessment supplies (i.e., full kit for some, additional recording sheets for those for which the laboratory already has access to main testing materials).
b. Train Professional Research Assistant (PRA) on administration of cognitive and behavioral measures where needed. The PRA is already fully trained, but may need refresher with some measures.

Progress on Task 2:
a. Behavioral assessment supplies were purchased as planned, within the first month of award commencement.

b. The PRA working on the study (Erika Shelton) was trained on all behavioral measures in Months 1 and 2 of the award (training in Month 1, with additional practice on volunteers around the lab in Month 2). Language and communication are being measured outside the MEG using the Preschool Language Scale-5 (PLS-5), the Peabody Picture Vocabulary Test (PPVT-III), and the Token Test for Children, 2nd Edition (TTFC-2). To determine nonverbal cognitive abilities, participants also complete the Leiter International Performance Scale-Revised. These measures were chosen as ones that can be completed by both verbal and nonverbal participants. In anticipation of the PI change in Year 2, the graduate students who will be working with Dr. Hepburn have begun training on these measures in the last 2 months of Year 1.

Task 3. **Participant Recruitment/Enrollment, Months 1-22**

a. Coordinate recruitment strategy with Dr. Susan Hepburn and staff.

b. Send out recruitment letters to patient database and various agencies such as local community centered boards, especially Developmental Pathways.
c. The PRA will conduct eligibility screening and informed consent process.
d. When ADOS/ADI-R information are not available for participants in autism groups, qualifiying interviews for qualifications are conducted by Dr. Hepburn and/or her staff.
e. For eligible participants, videos and MEG brochure will be sent to parents to familiarize participants with MEG procedures.
f. Projected quarterly enrollment: we plan to enroll 3 subjects per month into the study, so projected quarterly enrollment is 9 subjects per quarter (45 enrolled by the end of the first quarter in Year 2).

Progress on Task 3:
a. Recruitment in the first months of the study was slow, so efforts were increased to improve enrollment. To facilitate this, the study was added to the campus listing of clinical trials, for which a campus-wide email is sent out every 2 weeks. In addition, study flyers were handed out at various events in the community, including the Autism Speaks Walk in Denver and training workshops on campus targeted to parents of children with autism spectrum disorders.

b. As part of increased recruitment efforts mentioned in (a), Dr. Hepburn began sending out individualized letters to parents of potential research subjects who had either participated in other research programs on campus, or who had worked with Dr. Hepburn in a clinical capacity and had expressed interest in research. This was in addition to placing study advertisements on community-centered boards.

c. The study PRA (Erika Shelton) has been completing telephone eligibility screening with all interested parents. Either Erika Shelton, Dr. Hepburn, or Dr. McFadden have been completing the informed consent process with all eligible participants.

d. Dr. Hepburn and her clinical staff have been completing ADOS/ADI-R interviews with any potential participants who have not completed those measures with a qualified clinician within the past 3 years.

e. A video explaining all procedures used in the study was created in Months 1-3 to be sent to potential study subjects. This video features Dr. McFadden and a volunteer individual (a teenager with an autism spectrum disorder), who has previously completed research studies with Dr. Hepburn and consented to be in both the study and the video. This video shows Dr. McFadden explaining all study procedures and showing potential subjects what the MEG lab looks like. Additionally, the video depicts our volunteer study subject completing an example of the cognitive tests and MEG recording. The study subject narrates much of the video, explaining to parents and potential subjects what to expect. The video is 6 minutes long and is sent to parents (via an online link) who call about the study so that they can watch it with their child and determine if they are interested in participating. One of the goals of the study was to identify effective methods of increasing subject comfort with MEG recording prior to their study visit. The video has shown to be effective, as many kids have commented that they remember Dr. McFadden and the lab from the video, which appears to greatly increase comfort levels. In addition to the video link, a MEG brochure is also sent to parents prior to study participation.

f. As of now (Month 11 of Year 1), the study has been completed by 5 subjects in the nonverbal ASD group, 2 subjects in the verbal ASD group, and 1 subject in the control group. We currently have 1 subject in the verbal ASD group consented and ready to begin study participation, in addition to 3 subjects in the verbal ASD group currently completing the eligibility procedures.

Recruitment was slow in the first few months of the study, but efforts to increase recruitment have been successful and we are receiving an increased number of phone calls from interested parents. The study is now running smoothly, so we anticipate being able to complete the study with the originally planned 15 subjects per group by the end of Year 2.

Task 4. **MEG Scans and Behavioral Assessments, Months 2-22**

a. MEG training (1 lab visit, if needed)
b. MEG scans during auditory and language tasks (1 lab visit)
c. Behavioral assessments (1-2 lab visits over 2 month period)

Progress on Task 4:

a. We have discovered that a separate training visit prior to MEG scanning has not been necessary if the subjects are shown the MEG training video before their study visit. All parents are sent the link to the video, in addition to a brochure on MEG procedures, prior to coming in to the lab. Study subjects have commented that this has reduced anxiety surrounding the study visit, as they feel familiar with the surroundings before they come in.

b. MEG scans have been successfully completed by all study subjects thus far. We have had 2 subjects who have not been able to pay attention sufficiently during the language task, due to impaired cognitive abilities. However, we were able to successfully record MEG data during the auditory task for both of these subjects.

c. We have been able to complete the behavioral assessments during the same lab visit as the MEG scan for most subjects so far. Some of the children in the nonverbal group have been unable to complete all behavioral measures due to cognitive impairment, but we have been successful in obtaining at least some cognitive/language measures for all but 1 participant so far.

Task 5. **Interim Analyses, Months 10-16**

a. Interim statistical analyses of data obtained from MEG and behavioral/cognitive assessments. This will be done as each outcome measure reaches a minimum of 8 completed participants per group.
b. Annual reports will be written.

Progress on Task 5:

a. As we have not yet reached 8 completed participants in any of the groups, interim statistical analyses have not begun. However, we have completed testing on the motion correction hardware that was created for this project.

<u>Motion correction testing:</u>

A magnetic dipole phantom was used to test the efficacy of applying our newly developed motion correction technique to MEG data in which the subject moves repeatedly during data recording. During recording, the phantom was moved, such that there were 5 shifts in position throughout the MEG recording. The technique we have developed (with MEG lab electrical engineer Peter Teale and MEG lab physicist Eugene Kronberg; see circuit design in Appendix) involves the use of high-frequency signals outside a range that would be observed from measured brain activity. By localizing these signals throughout the recording, we are then able to correct for movement occurring during recording. In Figure 1, below, the data were filtered to remove these high-frequency signals (after using them for localization of movement). Data were then corrected for movement using the calculations derived from the high-frequency signals. The average MEG waveform is shown before motion correction (panel A) and after motion

correction (panel B). The scale of the signal is the same in both panels A and B. Noise in the recording created by subject movement can result in a cancellation of signal. The larger amplitude shown in Panel B demonstrates that we experience less signal loss when using motion correction than we would if we did not incorporate motion correction. As such, given that the subjects in this project are likely to move during MEG recording, the use of our motion correction technique will result in higher quality data than would be possible without this technique. Furthermore, goodness of fit and correlation, as measured by use of the phantom, were increased when applying motion correction compared to when we did not apply the correction.

Panel A (before correction)

Panel B (after correction)

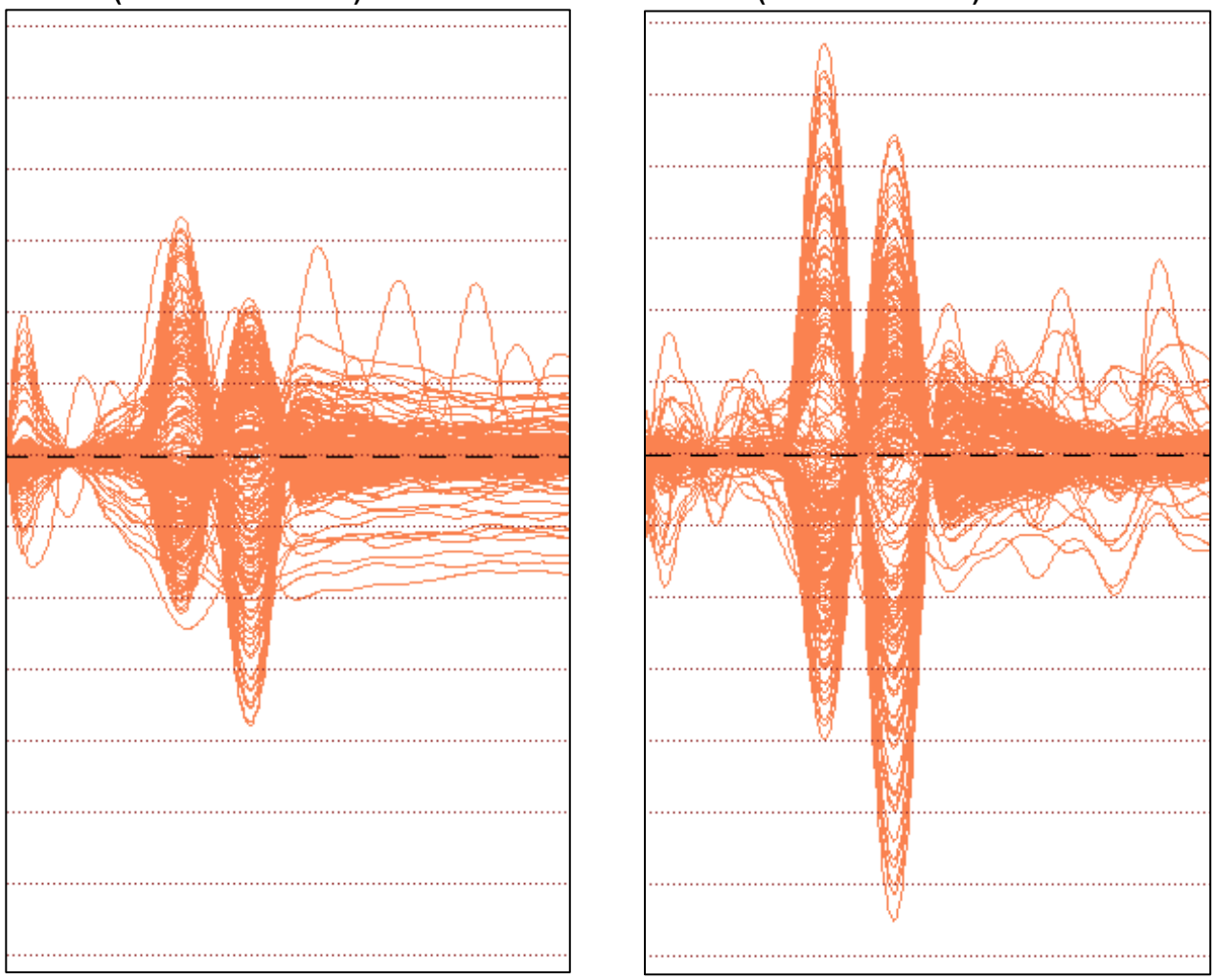

Figure 1. Demonstration of decreased signal loss due to subject movement when using movement correction (Panel B) compared to not using movement correction (Panel A).

b. The annual report for Year 1 has been completed.

Key Research Accomplishments

1. Development and testing of a motion correction system for the MEG system used in the current study (Magnes 3600 Whole Head Magnetoencephalograph).

2. Development of a video for potential study subjects explaining procedures used in the study, focusing on the MEG component.

Reportable Outcomes

1. Development of technique for correction of subject movement during MEG recording. We may disseminate this design in the future for other labs to use.

2. Development of a video explaining what it's like to participate in the MEG study, which is sent to parents of potential study participants. While this video was designed and created with the current study in mind, we kept it broad enough that it can also be used for future studies. For example, we show a brief demonstration of one of the cognitive tasks used in the current study, but we say that this is an example of one of the tasks that subjects may be asked to complete. Similarly, the MEG tasks demonstrated in the video are the ones used in the current study, but we mention in the video that subjects may be asked to complete different tasks, depending on the study in which they are participating.

3. Poster presentation at the American College of Neuropsychopharmacology (ACNP) 2013 annual meeting in Hollywood, FL (December, 2013):
McFadden KL, Steinmetz SE, Hepburn S, Tregellas JR, Rojas DC. Steady-state gamma-band responses in children with autism spectrum disorders during an auditory oddball task.

While this presentation did not include data from the current project (data collection had only just begun at the time of the conference, due to sequester-related delays in award start date), the current project was discussed as an extension of this work.

Conclusion

A main goal of this project is to determine a means of measuring receptive language in a more sensitive and accurate way than can currently be achieved in children with autism spectrum disorders (ASD) who are nonverbal, and neural markers have the potential to be more effective than behavioral measures in this population. Results from this project can inform development of future studies assessing speech intervention outcome in nonverbal children with ASD using neural markers. This project will significantly contribute to the knowledge of underlying neurophysiology in language processing in ASD. At this point in the study, we have completed a number of key goals. First, we have successfully developed a technique to correct MEG data for subject movement during recording. This correction reduces signal loss due to movement, resulting in higher quality data. Given that the population we are studying in this project, particularly those with greater cognitive impairment, are likely to exhibit some movement during recording, the ability to correct for that movement is important for the project. Additionally, we have created a video to send to parents of potential study participants, which describes the study procedures. This video was designed to increase subject comfort; based on reports from subjects who have completed the study, this approach appears to be successful in reducing anxiety surrounding the study visits. We have completed data collection with 5 subjects in the nonverbal ASD group, 2 subjects in the verbal ASD group, and 1 subject in the control group. We currently have 1 subject in the verbal ASD group consented and ready to begin study participation, in addition to 3 subjects in the verbal ASD group currently completing the eligibility procedures. The study is running smoothly, so we anticipate being able to complete study procedures with the originally planned 15 subjects per group by the end of Year 2.

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3.Rojas DC, Maharajh K, Teale P, Rogers SJ. Reduced neural synchronization of gamma-band MEG oscillations in first-degree relatives of children with autism. BMC Psychiatry 2008;8:66.
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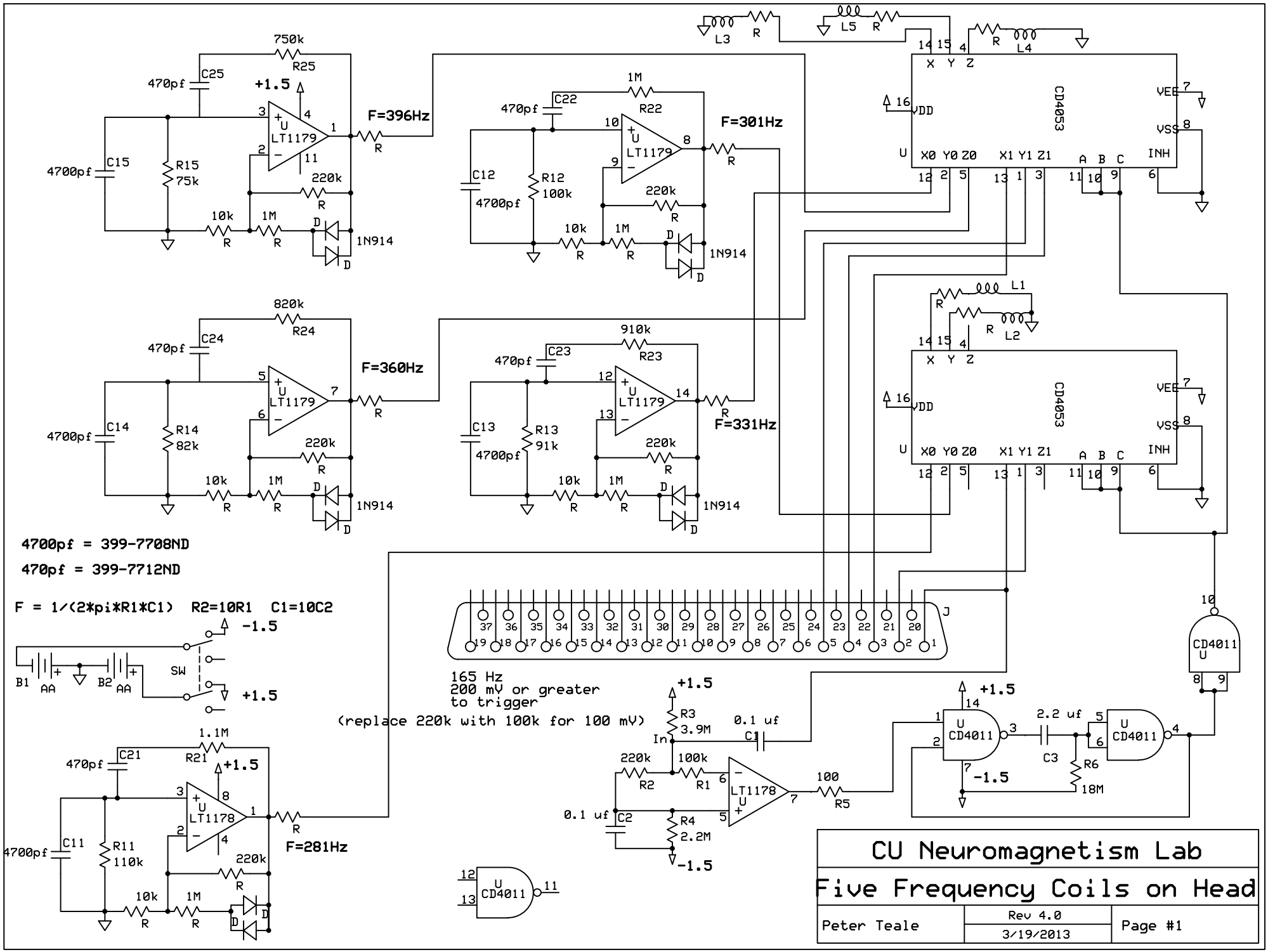
Appendix

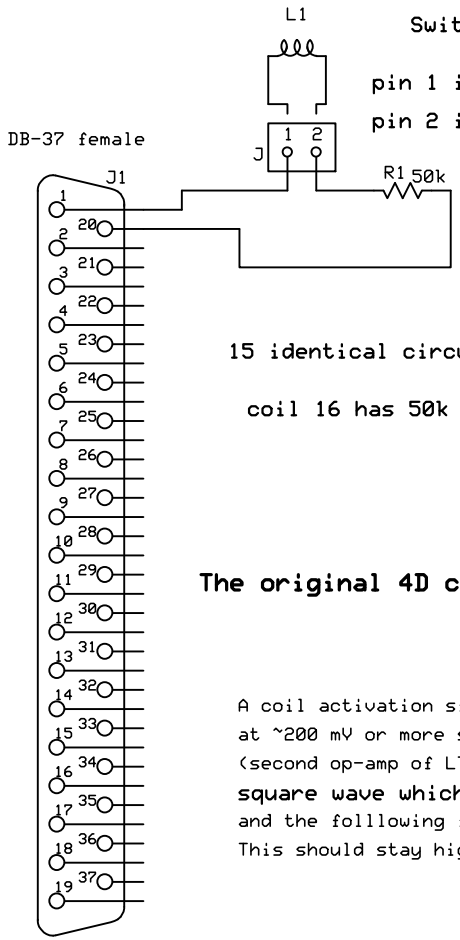
The following pages depict the circuit board design for the head coil interface developed for this project by Peter Teale, the electrical engineer for the Magnetoencephalography Laboratory. This device is currently being used during data collection to allow for movement correction during data analyses.



CU Neuromagnetism Lab

Five Frequency Coils on Head

Peter Teale	Rev 4.0 3/19/2013	Page #1



Switchcraft Micro D Jax P/N MDPC2A

pin 1 is the tip

pin 2 is the sleeve on the jack

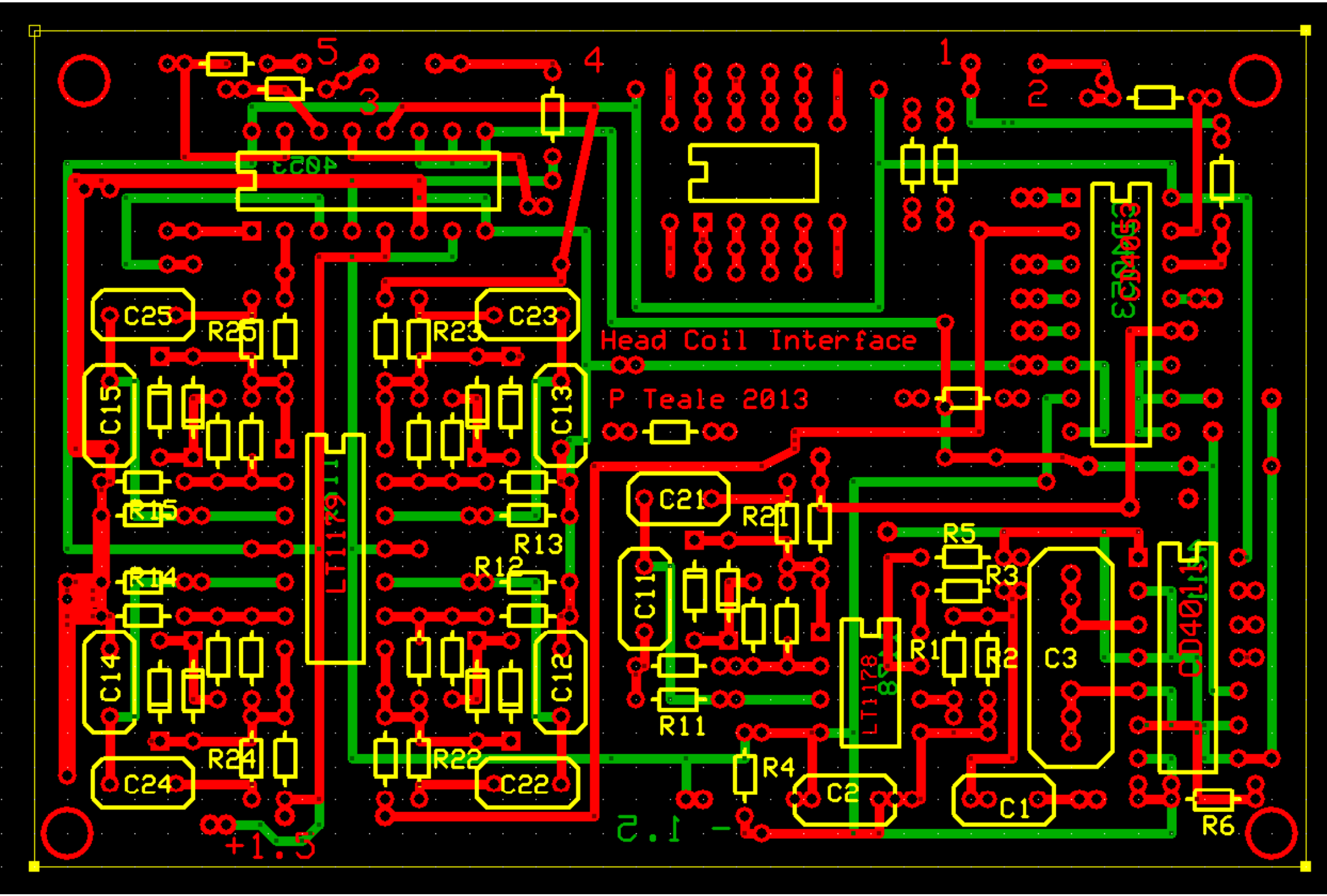
15 identical circuits

coil 16 has 50k resistor removed (2012)

The original 4D coil interface box has 50k resistors in series with each coil

A coil activation signal (165 Hz sine)) from coil 1
at ~200 mV or more should cause the open loop op-amp
(second op-amp of LT1178) to produce a rail-to-rail
square wave which will set the monostable output low (pin 4 of CD4011)
and the folllowing inverter output high (pin 10 of CD4011).
This should stay high for about 4 seconds.

CU Neuromagnetism Lab		
Five Frequency Coils on Head		
Peter Teale	Rev 4.0 4/19/2013	Page #2


Head Coil Interface
P Teale 2013
C25
R25
C23
R23
C15
C13
R15
R13
R14
R12
C14
C12
R24
R22
C24
C22
C21
R21
C11
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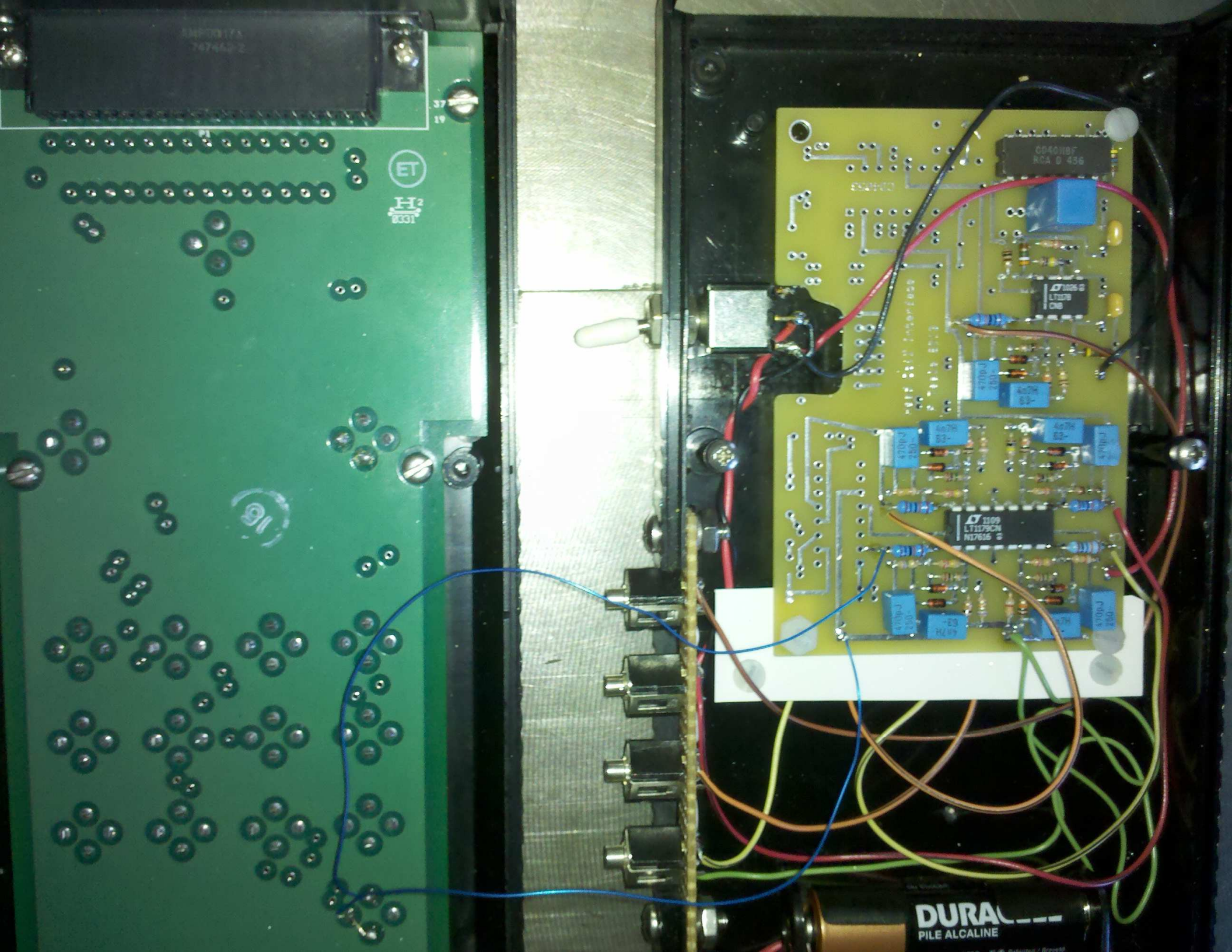
R1
R2
C3
R4
C2
C1
R6
LT1128
CD4013
+1.5


CD4011BF
RCA D 436
LT1178
CN8
LT1179CN
N17515
DURA
PILE ALCALINE